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May 26, 2015

Evaluation of the New York State Human Papilloma Virus (HPV) Proficiency Test

April 2015

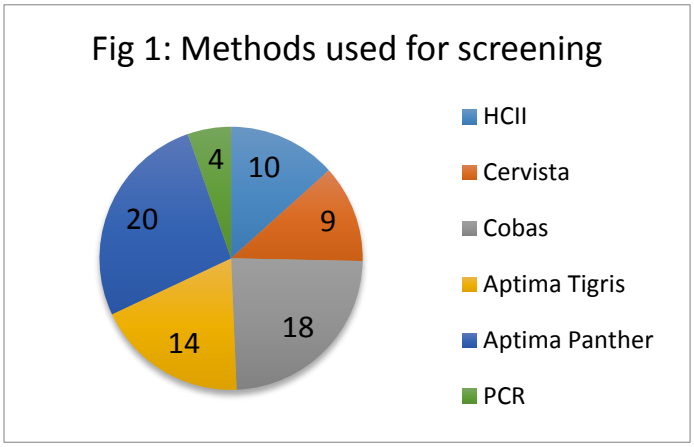
Dear Laboratory Director:

Here is the summary and evaluation of the New York State Proficiency Test for human papilloma virus (HPV) from April 2015. A report with your laboratory's score and grade will be sent separately to you by regular mail. Five vials (HPV086 – HPV090) containing cervical cells derived from actual patients in PreservCyt® medium were sent out to every permitted laboratory on April 7th, 2015, and the due date for submitting the test results was April 27th, 2015. Each correct answer received 20 points, and an incorrect one zero points. The passing threshold was set at 80 points (80 percent) for the entire test event. Answers could be provided in three categories, Positive (Pos), Negative (Neg), or Low Positive (LoPos) for **high-risk** HPV screening. **Please note:** only samples that tested positive for one or more of the known or suspected high risk genotypes should have been reported as screen positive. Laboratories that perform genotyping were also asked to provide those results. In addition, we asked that you include the raw data with your results, i.e. RLU/CO values from Hybrid Capture®, FOZ values from Cervista®, Ct values from the Roche Cobas®4800 method, or S/CO ratios from the Aptima® methodology, though this information was not used for grading.

Cytology smears were prepared and evaluated in-house from each of the samples. Samples HPV087 and HPV089 were diagnosed as "Satisfactory for evaluation", "Negative for intraepithelial lesion or malignancy" (NILM). Sample HPV090 was evaluated as "Satisfactory for evaluation" with "Low-grade squamous intraepithelial lesion" (LSIL), and finally, samples HPV086 and HPV088 presented with abnormal cells showing clear evidence of koilocytosis and were both diagnosed as "Satisfactory for evaluation" with "LSIL (Low-grade squamous intraepithelial lesion) consistent with HPV infection". These diagnoses were consistent with the HPV proficiency test results for those samples (see below).

Screening Results (Tables 1a, b)

A total of 76 laboratories received samples, and 75 submitted valid answers by the due date. Ten laboratories (13.3%) used the Hybrid Capture® method, 9 laboratories (12.0%) used the Cervista® method, 22 laboratories (29.3%) used a polymerase chain reaction based method (18 Cobas®4800 and 4 a Laboratory Developed Test) and 34 laboratories (45.3%) used the Aptima® method (14 laboratories used the Tigris instrument and 20 laboratories used the Panther instrument) (Fig 1).



With the exception of one PCR laboratory for two samples, all laboratories agreed with the respective consensus for each sample for an overall concordance of 373/375 (99.5%) results across all samples and all methods. This one laboratory reported samples HPV087 and HPV089 as positive, presumably on the basis of finding the low risk genotypes 54 and 81, respectively, in these samples. Please note, although low-risk genotypes may be detected in a sample, when there are no concurrent high risk genotypes present the result should be reported as negative.

Table 1a: Screening results, all methods combined (75 laboratories)

	HPV086	**HPV087**	**HPV088**	**HPV089**	**HPV090**
All methods					
Total	75	75	75	75	75
Negative	0	74	0	74	0
Positive	75	1	75	1	75
Low Positive	0	0	0	0	0
Indeterminate	0	0	0	0	0
% Negative	0.0%	98.7%	0.0%	98.7%	0.0%
% Positive	100.0%	1.3%	100.0%	1.3%	100.0%
% Low Positive	0.0%	0.0%	0.0%	0.0%	0.0%
% Indeterminate	0.0%	0.0%	0.0%	0.0%	0.0%
Consensus	**POS**	**NEG**	**POS**	**NEG**	**POS**

Table 1b: Screening results, by method

	HPV086	HPV087	HPV088	HPV089	HPV090
Hybrid Capture®					
Total	10	10	10	10	10
Negative	0	10	0	10	0
Positive	10	0	10	0	10
Low Positive	0	0	0	0	0
Indeterminate	0	0	0	0	0
% Negative	0.0%	100.0%	0.0%	100.0%	0.0%
% Positive	100.0%	0.0%	100.0%	0.0%	100.0%
% Low Positive	0.0%	0.0%	0.0%	0.0%	0.0%
% Indeterminate	0.0%	0.0%	0.0%	0.0%	0.0%
Consensus	**POS**	**NEG**	**POS**	**NEG**	**POS**

	HPV086	HPV087	HPV088	HPV089	HPV090
Cervista®					
Total	9	9	9	9	9
Negative	0	9	0	9	0
Positive	9	0	9	0	9
% Negative	0.0%	100.0%	0.0%	100.0%	0.0%
% Positive	100.0%	0.0%	100.0%	0.0%	100.0%
Consensus	**POS**	**NEG**	**POS**	**NEG**	**POS**

	HPV086	HPV087	HPV088	HPV089	HPV090
Cobas® 4800					
Total	18	18	18	18	18
Negative	0	18	0	18	0
Positive	18	0	18	0	18
% Negative	0.0%	100.0%	0.0%	100.0%	0.0%
% Positive	100.0%	0.0%	100.0%	0.0%	100.0%
Consensus	**POS**	**NEG**	**POS**	**NEG**	**POS**

	HPV086	HPV087	HPV088	HPV089	HPV090
Aptima®					
Total	34	34	34	34	34
Negative	0	34	0	34	0
Positive	34	0	34	0	34
% Negative	0.0%	100.0%	0.0%	100.0%	0.0%
% Positive	100.0%	0.0%	100.0%	0.0%	100.0%
Consensus	**POS**	**NEG**	**POS**	**NEG**	**POS**

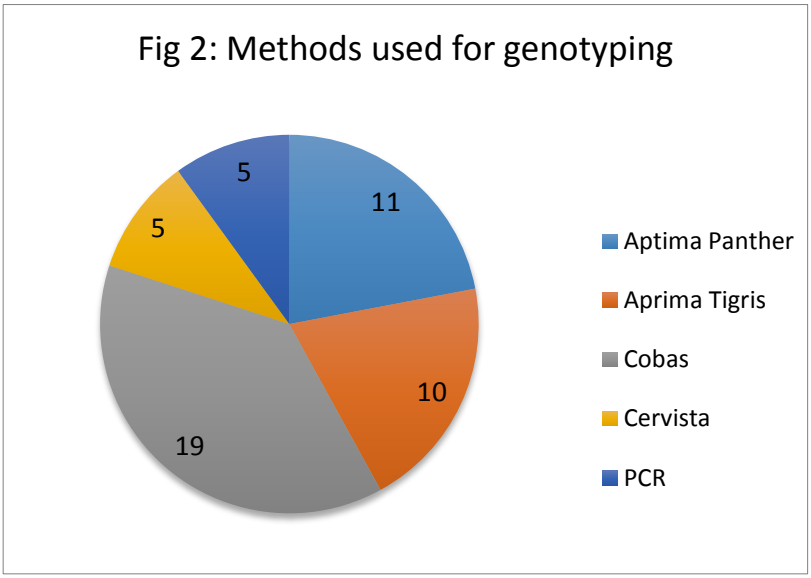
Table 1b, cont.

	HPV086	HPV087	HPV088	HPV089	HPV090
PCR (LDT)					
Total	4	4	4	4	4
Negative	0	3	0	3	0
Positive	4	1	4	1	4
% Negative	0.0%	75.0%	0.0%	75.0%	0.0%
% Positive	100.0%	25.0%	100.0%	25.0%	100.0%
Consensus	**POS***	**NEG***	**POS***	**NEG***	**POS***

*Based on all laboratory consensus

Genotyping (Table 2)

Laboratories that routinely determine HPV genotypes were also asked to submit those results. Fifty genotyping results derived with various methods were submitted. Of those, 21 (42%) were from the Aptima® method, 19 (38%) from the Roche Cobas®4800 method, 5 (10%) from the Cervista®16/18 method, and 5 (10%) from a laboratory-developed PCR based method. Two of these genotyped by DNA sequencing, one laboratory followed with RFLP, another laboratory performed capillary electrophoresis, and the fifth laboratory used a linear array panel for its genotyping method. (Fig 2). However, since not every method equally detects and/or discriminates every genotype and because the samples represent mixtures of patient samples, the genotyping results were not graded. You must, however, compare your results to that of the majority, shown in Table 2, and investigate any discrepancies.



For the screen positive samples HPV086 and HPV088 there was almost unanimous agreement that they contained the HPV16 genotype, whereas only less than half the laboratories also detected HPV18 and/or 45 in sample HPV086, and only about 60% of laboratories also

detected HPV18 and/or 45 in sample HPV088 (assuming that if a laboratory tested for HPV16 it also tested for HPV18/45). In contrast, essentially all laboratories detected both HPV16 and 18 and/or 45 in sample HPV090. Only a few laboratories also reported the presence of other non16, non18 genotypes, which is surprising since all 19 Cobas users reported all three channels as positive (Ct <40, see Figure 3).

Responses from the Roche Cobas®4800 method indicated that all three screen positive samples, HPV086, HPV088 and HPV090, contained a mixture of the HPV high-risk genotypes 16, 18 and one or more of the other high risk genotypes included in the Roche assay, based on a positive signal in all three channels on the instrument.

The five laboratories that genotyped with a laboratory developed PCR based method were consistent in detecting the HPV genotype 16 in all of the screen positive samples, with the exception of one laboratory that submitted genotype HPV66 for sample HPV088. Although HPV66 is considered high risk and part of the panel of most assays, we wonder whether this laboratory made a data entry typographical error, meaning to enter 16 instead of 66. In contrast, only two and three, respectively, of five laboratories also detected HPV18 in the three positive samples. Finally, two laboratories claim to have detected high risk genotypes in the screen negative samples HPV087 and HVP089, but did not report these samples as positive in their screening assay, suggesting that their results are not internally consistent.

In conclusion, while the ability to detect HPV16 was consistent, there seemed to be substantial inconsistencies in detecting HPV18. While the exact reasons for this discrepancy are unclear, it is possible that HPV18 was present at somewhat lower levels than HPV16, especially in samples HPV086 and HPV088, below the analytical sensitivity of several LDTs.

Table 2. Genotyping results (49 laboratories)

Overall Results	**HPV086**	**HPV087**	**HPV088**	**HPV089**	**HPV090**
Total 16	49	3	49	3	50
Total 18 and/or 45	18	2	29	2	45
Total 45	1	0	0	0	2
Total other genotypes	2	3	1	3	1
Results by method					
Total 16 Aptima	21	1	21	1	21
Total 16 Roche	19	2	19	2	19
Total 16 Cervista	4	0	5	0	5
Total 16 Other Methods	5	0	4	0	5
Total 18/45 Aptima	4	1	6	1	19
Total 18 Roche	12	1	18	1	18
Total 18 Cervista	0	0	2	0	5
Total 18 Other Methods	2	0	3	0	3

Raw data

Figures 3 and 4 show the distribution of the raw data from the different instruments. While none of the assays is strictly quantitative, these data nevertheless allow a comparison between your results and those of your peers. For example, two laboratories apparently did not find HPV18 in sample HPV086 by their Roche Cobas method (see Figure 3F). Similarly, there seemed to be some discrepancy in the APTIMA HPV18/45 results between laboratories for samples HPV086, HPV088 and HPV090 (Figure 4D). We suggest that if your result is part of the minority group to reexamine your data.

Figure 3: Screening data by method

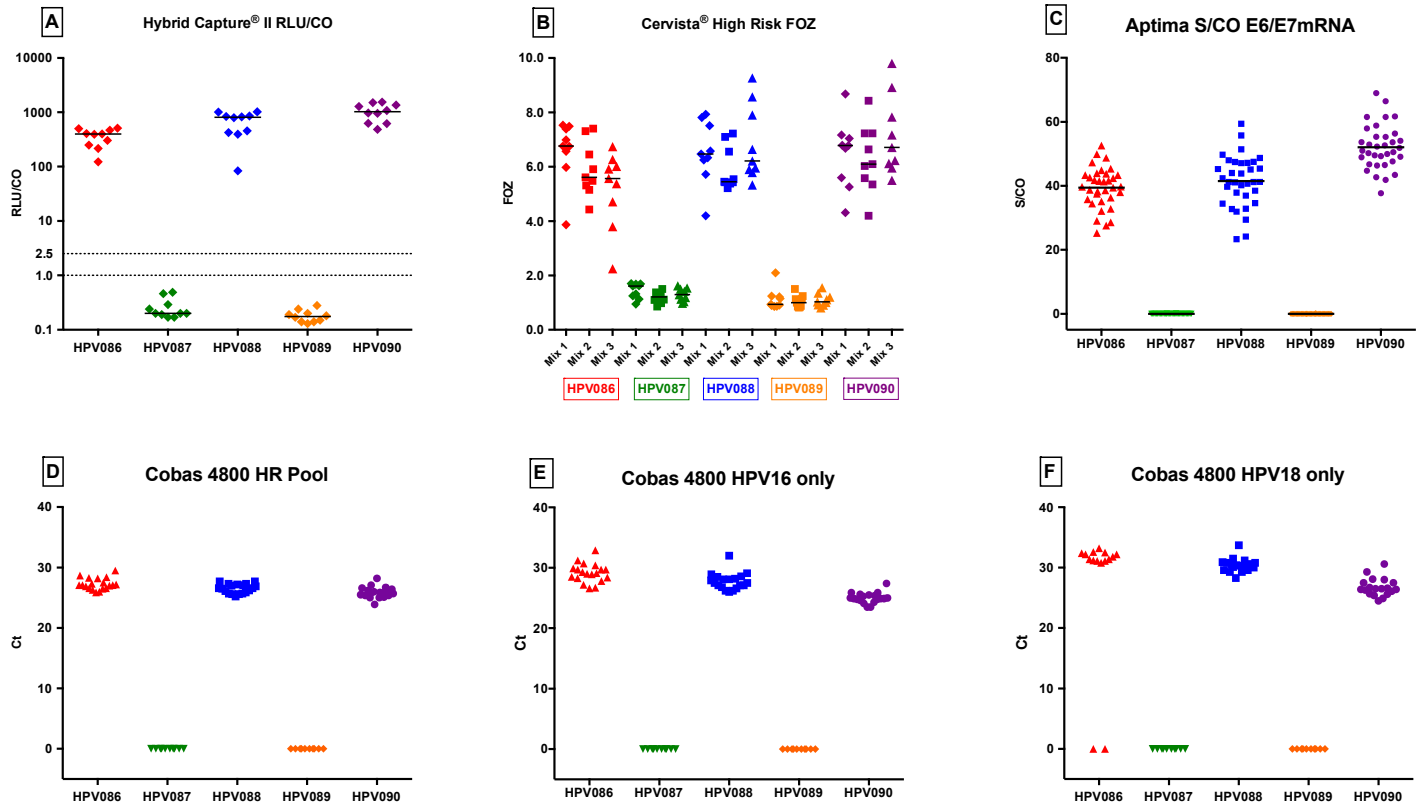
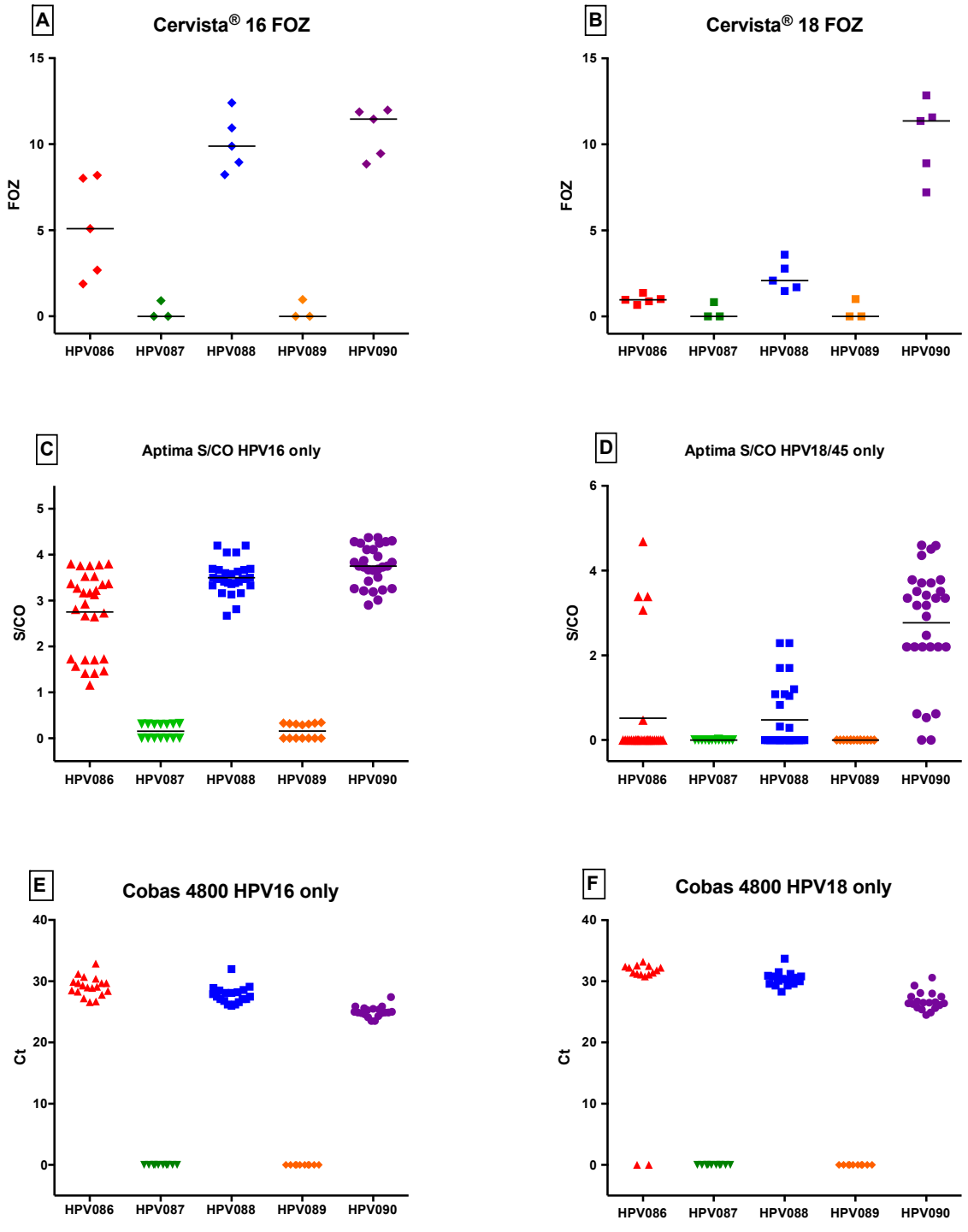


Figure 4: Genotyping data by method



Conclusions

The high overall screening consensus of 99.5% achieved for this proficiency test was excellent and the results were consistent with the cytologic features of the samples. As for the genotyping results, while there was general consensus for the presence of HPV16 in the three screen positive samples, there was significant disagreement as to the presence of HPV18 in samples HPV086 and HPV088.

Finally an important reminder regarding the data submission process: Be sure your results are submitted. If results are saved but **not submitted**, they will be graded as an administrative **fail** and put your lab at risk for an unsuccessful performance. Also, be sure to read the instructions on how to report your results in the cover letter included in the next sample shipment.

Tentative schedule for the 2015 New York State HPV proficiency tests:

Mail-out Date	Due Date
October 13	November 2

For questions, comments or suggestions regarding this PT event please call or e-mail:

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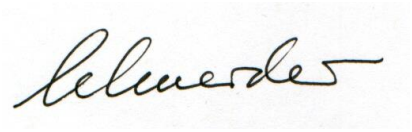

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